Gravitropic Responses of Partially Decapitated Corn Coleoptiles with and without Applied [14C]Indoleacetic Acid[1]

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ABSTRACT

The curvature of corn seedling (*Zea mays* L. Mo17 × B73) coleoptiles which had been half-decapitated and supplied with [14C]indoleacetic acid (IAA) (3.2 micromolar, 51 milliCuries per millimole) was determined during a 3-hour period of gravitational stimulation. Curvature of such half-decapitated coleoptiles was found to be similar in rate and extent to that of intact coleoptiles responding to gravity. Gravitational stimulation was accomplished by reorienting seedlings to a horizontal position, either up or down with respect to the removed half of the coleoptile tips.

The first set of experiments involved placing aluminum foil barriers along one of the two cut surfaces to restrict the movement of IAA into tissues. The initiation and extent of curvature of these half-decapitated coleoptiles was dependent upon the orientation of the removed half-tip and the accompanying barrier. The distribution of radioactivity from [14C] IAA after 3 hours indicated that the specific lateral movement of label was also dependent upon orientation of the removed half-tip of the coleoptile. A specific movement to the lower side of approximately 14% of the total recovered radioactivity was found in coleoptiles in which the [14C]IAA was supplied across a transverse cut surface. In contrast, specific movement of only 4% was found for application across a longitudinal cut surface.

A second series of experiments was conducted using 1.0 and 3.2 micromolar [14C]IAA (51 milliCuries per millimole) supplied to half-decapitated coleoptiles without inserted barriers. The 3.2 micromolar concentration adequately replaced the removed coleoptile half-tips in terms of straight growth, but it did not result in as much curvature as shown by coleoptiles of intact seedlings. The 1 micromolar concentration was not adequate to replace the removed half-tip in straight growth, but resulted in gravitropic curvature nearly as great as that produced by the higher concentration.

The data presented here suggest that strong auxin gradients are not produced in response to gravity stimulation based on the recovered radioactivity from [14C]IAA. However, it is evident that auxin is required for the development of normal gravitropic responses. It is possible, therefore, that an important early role of this movement is not to cause a large stimulation of growth on the lower side but to decrease growth on the upper side of a gravitropically responding coleoptile.

In an early attempt to relate changes in growth patterns during gravitational stimulation to changing hormone levels, Navez and Robinson (12) accurately documented the curvature of *Avena* coleoptiles. They concluded that the curvature of the coleoptiles was due to an increase in elongation rate on the lower side and a decrease on the upper side. Results of experiments involving collection of diffusible auxin from partially split coleoptile tips

after various periods of gravitational stimulation led them to believe that auxin was moving laterally in the tip, leading to the observed stimulation of growth on the lower side. Though the diffusate experiments were consistent with the asymmetric growth pattern observed, there was no proof that the asymmetry was caused by a change in auxin levels.

It is now well established that IAA is transported laterally in coleoptile tissue, both in intact coleoptiles (15) and in segments (10). This fulfills an essential feature of the Cholodny-Went theory that requires auxin to be transported laterally. Much controversy still exists over the complete validity of this theory. Recent criticisms of it by Firn and Digby (7) and Trewavas (16) question the significance of this lateral transport observed in coleoptiles. Their main contention is that the magnitude of the lateral transport of IAA is not sufficient to account for the observed increase in growth rate on the lower side of the coleoptile.

Though much work has been done on gravitropism, no definitive answers have come forth to explain the mechanism of action of IAA or its role in the response. Trewavas (16) has suggested that the main function of growth regulators such as IAA is to support continued growth but not to control growth rate or distribution. If this is true, it is expected that as long as sufficient IAA is present to maintain growth, normal gravitropic curvatures will occur irrespective of the presence or absence of the lateral transport of IAA.

The investigations reported here have sought a better understanding of the roles of auxin in the gravitropic responses. We have observed that the rate of curvature of decapitated, IAA-treated coleoptiles is 2 to 3 times slower than that of intact coleoptiles (11). In contrast, half-decapitated, IAA-treated coleoptiles responded to gravity in a fashion that closely paralleled the response of intact coleoptiles on whole seedlings. For this reason, both the curvature and the distribution of label from IAA was followed in half-decapitated coleoptiles.

MATERIALS AND METHODS

Corn seeds (*Zea mays* L. Mo17 × B73) were soaked for 12 h in running tap water (30 ± 1°C), and were placed embryos up on moist paper towels. After 24 h, uniform seedlings were selected and planted in individual glass vials (2.5 × 10 cm) containing moist vermiculite. Seedlings in individual vials were held vertical in a special plastic chamber (300 × 300 × 25 cm) lined with moist paper towels and grown under red light (0.1 μw m^{-2} integrated over the range 600–725 nm). Experiments performed 60 h after completion of the 12-h soak.

IAA solutions were prepared in citrate-KOH buffer (20 mM, pH 5.65) as was the stock agar solution for each experiment. IAA solutions were applied to corn seedlings in the form of agar blocks (3·× 3 × 1.2 mm). Final agar concentration for each IAA solution was 1.5%.

Coleoptiles were half decapitated by bisecting the tip with a 3-

mm cut between the vascular bundles, then cutting transversely through one of the bundles to remove one-half of the tip. An agar block containing either 0.0, 1.0, or 3.2 μM IAA was applied immediately to these cut surfaces. To apply gravitational stimulation to the seedlings, the vials were reoriented 90° so that the longitudinally cut side of each coleoptile was up or down. In all experiments, a control set of intact seedlings was given the same gravitropic stimulation. Three-h gravitational stimulations were selected because the intact coleoptiles showed a nearly constant rate of curvature during this time interval. All decapitation procedures and gravitropic measurements were carried out in a large (8 m^3) humid chamber (95–100% RH). Photographs were taken every 30 min during a 9-s green-light exposure and degrees of curvature were determined from tracings of the projected images of the coleoptiles. The curvature of an individual coleoptile was determined at the intersection of two tangents, one to the node and one to the tip. This provided a measurement of curvature of the coleoptile alone and not of the whole seedling.

The distribution of radioactivity from [^{14}C]IAA in gravitropically stimulated seedlings was determined after applying it to partially decapitated coleoptiles for 3 h. The [^{14}C]IAA, obtained from New England Nuclear, was purified by two isocratic solvent passes through the 15-cm C-18 Ultrasphere column of a Beckman HPLC. The first pass consisted of 40% acetonitrile in water adjusted to pH 3.5 with acetic acid. After collecting and reducing the volume of appropriate fractions, the sample was further resolved in a single peak with a solvent pass of 48% methanol in water at pH 3.5.

A set of trials was conducted with 3.2 μM IAA in the donor blocks. Barriers of 4 × 4 mm aluminum foil, when used, were inserted in one of four different positions. Each barrier was placed on the vertical or horizontal cut face of a half-decapitated tip. In all cases, barriers extended beyond the cut surface (<1 mm) into the remaining tip tissue. After 3 h, each block was removed, placed in an individual scintillation vial to which 10 ml of scintillation fluid was added, and radioactivity was counted. Each batch of scintillation fluid consisted of 1 liter of toluene, 0.5 liter of Triton X-100, and 4 g of 2,5-diphenyloxazole. ^{14}C activity was assumed to be [^{14}C]IAA based on previous findings by other workers with etiolated *Zea mays* coleoptiles.

In the barrier experiments, each coleoptile was immediately excised at the node and halved by splitting between the vascular bundles. Individual halves were placed in separate scintillation vials containing 5 ml of methanol. The tissue was extracted in methanol for 18 to 20 h at −4°C after which the methanol was quickly evaporated by a stream of air and scintillation fluid was added. All samples were counted in a Packard Tri-Carb model 3375 liquid scintillation spectrometer. Counting efficiency for ^{14}C was 90% and quenching due to tissue samples was consistent at 15 ± 5%.

A second set of experiments involved half-decapitated coleoptiles without barriers. The same procedure was used except that two concentrations of IAA were employed, 1.0 and 3.2 μM. Coleoptiles were excised, split longitudinally, and each half was divided into equal apical and basal halves. The apical portion of each coleoptile half included the portion in contact with the agar block. In those experiments using 1 μM IAA, the amount of uptake was small. For this reason, similar tissue sections were pooled in the same vial. Extraction and counting were performed as in the barrier experiments.

RESULTS AND DISCUSSION

Gravitropic Responses of Coleoptiles with Barriers. Figure 1A illustrates the mean curvature of coleoptiles attached to intact seedlings over the 3-h period of gravitational stimulation. The rate of curvature reaches a maximum of 30 degrees h^{-1} before slowly decreasing. By 3 h the rate of curvature has decreased to
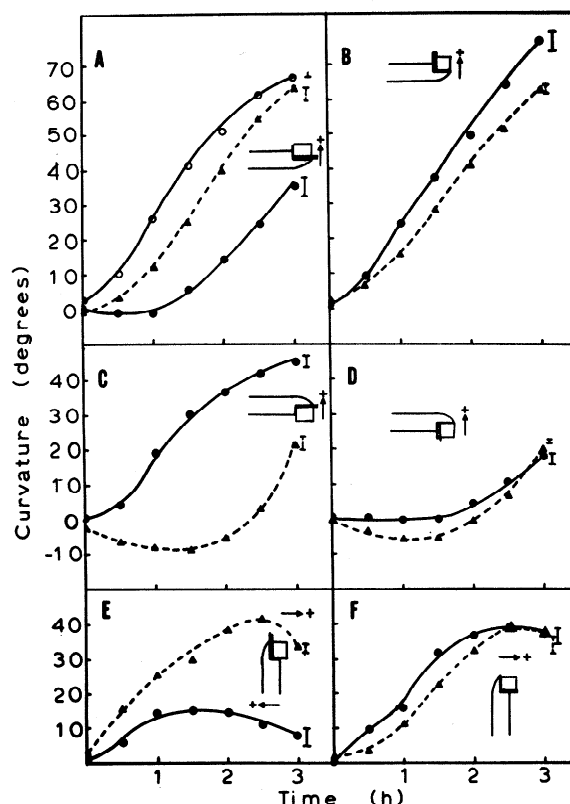

FIG. 1. Gravitropic responses of half-decapitated coleoptiles with IAA application restricted by barriers. Coleoptiles of intact corn seedlings were half-decapitated and supplied with 3.2 μM IAA in agar blocks containing 20 mM citrate-KOH buffer (5.65 pH). Aluminum foil barriers (cross-hatched) were inserted as shown. Sets of three coleoptiles, each given the same treatment, were placed horizontally for 3 h. Blank controls receiving no IAA (▲---▲) were included for each treatment. Also a set of intact coleoptiles (O——O) was given the same gravitational stimulation as a further control. Coleoptile curvature was determined every 30 min for the duration of the experiment. Arrows indicate the direction of curvature for each treatment. All points represent the means of four separate experiments. (SEM = ±2.1; the error bar by each curve represents the SD for individual means.)

about one-third of the maximum rate. The slowing of the curvature rate reflects the initiation of the autotropic response described by Firn and Digby (6). According to them, the decrease in rate of curvature that eventually leads to a straightening of the previously curved coleoptile is initiated by a decrease in growth on the lower side followed by an increase in growth on the upper side.

In comparison to the gravity-induced curvature of intact coleoptiles, there is a wide range of responses to half-decapitation and barrier position. The coleoptile orientation, barrier position, and the mode of IAA application will be referred to by the letter of the appropriate part of a figure. Parts A, C, and E of Figure 1 represent IAA application across a transverse cut of the coleoptile, while parts B, D, and F of Figure 1 represent application across a longitudinal cut of the coleoptile tip. It is evident (Fig. 1) that the position of the intact half of the coleoptile tip has an effect upon the resulting curvature.

Data presented in Figure 1E indicate that when 3.2 μM IAA is supplied only to the transverse cut surface of vertically held coleoptiles, a small curvature away from the removed half-tip results. This concentration must cause a slight stimulation of growth over that of the intact half. The curvature response is transient with the coleoptile returning toward the vertical posi-

tion in 3 h. The IAA supplied is nearly adequate to replace the removed half of the coleoptile tip, at least in terms of growth relative to the intact side. The response to the application of blocks containing no IAA shows a strong curvature towards the removed half-tip (Fig. 1E) indicating a need for auxin to support vertical growth. This does not necessarily mean that the IAA blocks are supplying the same amount of auxin as the intact half; wounding of the tissue probably has some effect on the growth response.

Figure 1C illustrates the curvature response when IAA is supplied through a transverse cut surface to the lower side of a gravitationally stimulated coleoptile. The initial curvature is similar to the intact response, but after 1 h the rate of curvature decreases faster than that of the intact coleoptile. For coleoptiles given the same treatment but not supplied with IAA, there is a small initial downward curvature followed by upward curvature after 1.5 h. Figure 1A shows the opposite IAA donor position in which the initiation of curvature is delayed for 1 h and then increases steadily over the next 2 h. The delay in curvature is probably caused by a transient stimulation of growth in the treated side as observed for vertical orientation (Fig. 1E). Timing of the initiation of curvature corresponds well with the decrease in stimulation of growth in vertical coleoptiles given the same decapitation, barrier treatment, and IAA supply.

The effects of IAA on half-decapitated coleoptiles when supplied only to the longitudinal cut surface are shown in Figure 1, B, D, and F. The data in Figure 1F illustrate the need for IAA to support normal vertical growth. The large curvature (40 degrees) towards the removed half-tip may have been due to a decreased growth rate on the barrier side. This would seem to be the case as the response to blocks containing no IAA is similar in timing and magnitude. Although the application of blocks containing IAA should increase the supply of auxin on the intact side, there appears to be only a slight stimulation in growth over the response to blocks containing no IAA. The increased supply of IAA on the intact side may be responsible, however, for the early initiation of curvature as compared to the blank controls. The initiation of recovery, delineated by a decrease in rate of curvature between 1.5 and 2.5 h, may be more complicated.

The slowing of the rate of curvature (Fig. 1F) may indicate that the barrier side has begun to adapt to the low endogenous level of IAA, resulting in an increased growth rate. Evans et al. (5) have shown that the adaptation process, called a spontaneous growth response (SGR) by them, requires about 3 h before initiation. It is possible that these partially decapitated coleoptiles may respond differently than isolated segments, accounting for an adaptation time of less than 2 h. In addition the intact side may become desensitized to the increased levels of auxin (11) contributing to the rapid decrease in curvature. An alternate possibility is that as the coleoptile curves a new gravitational vector is imposed on the tissue, altering the later response.

A comparison of the data represented in Figure 1B with the intact response (Fig. 1A) suggests that, additional IAA supplied through a longitudinal cut surface to the lower side has little effect on the rate of curvature for this tissue. That such an additional amount does enter the lower side is inferred from the data represented by Figure 1, E and F, in which the 3.2 μM IAA concentration caused a transitory stimulation of growth leading to the early curvature response of vertical coleoptiles. Distribution of radioactive label from [¹⁴C]IAA indicates that coleoptiles in this orientation (Table IB) take up more IAA than the corresponding vertical position (Table IF). The response during the first 1.5 to 2 h nearly parallels the intact response (Fig. 1A). Only after this initial period does the response deviate from that of intact coleoptiles by continuing at a linear rate rather than leveling off. The continued supply of additional IAA to the lower side, however, may be sufficient to override the increasing growth

rate on the upper side, which normally would lead to a decrease in the rate of curvature. This could account for the continued linear response rather than a decrease in curvature.

If the concentration of IAA were the controlling factor in mediating the gravitropic response, one might expect that supplying an additional amount of IAA would have stimulated a more rapid curvature than exhibited by intact controls. This clearly does not happen, indicating that the added IAA has no additional stimulatory effect. An alternative interpretation may be that, since the supply of IAA on the upper side is reduced due to the removed half-tip, the additional IAA merely replaces what normally would be transported laterally to the lower side.

Data in Figure 1D suggest that mechanisms other than auxin concentration may be controlling the growth rate on the upper side of gravitationally stimulated coleoptiles. One might expect from the results shown by vertical controls (Fig. 1F) that the transverse barrier would cause an initial positive (downward) gravitropic curvature in horizontal coleoptiles (Fig. 1D). The lack of such a positive curvature response implies that (a) either cells on the upper side do not respond to the additional IAA, (b) uptake into the upper side is limited, or (c) lateral transport of IAA is supplying sufficient IAA to the lower side to keep pace with growth on the upper side. Upward gravitropic curvature does begin after 1.5 h at a rate approximately one-third to one-half that of the intact rate. Blank controls given the same treatment (Fig. 1D) have a similar curvature response pattern. From the results of the radioactivity distribution experiments (compare Table I, B and D), it is not likely that the curvature (Fig. 1D) is due to the lateral transport of IAA alone. It seems feasible that the tissue on the lower side has adapted to the lower IAA level, becoming more sensitive to IAA. This would be particularly true for coleoptiles not supplied with IAA. Decapitated coleoptiles not supplied initially with IAA show an increased sensitivity to IAA within 3 h (11). This would account for the discrepancy between curvature observed after 1.5 h and the radioactivity measured after 3 h.

Distribution of radioactivity within the coleoptile indicates that lateral transport in these half-decapitated coleoptiles is not strong. Table I shows radioactivity as cpm and as per cent of total recovered from split coleoptiles after 3-h uptake periods, with barrier positions as indicated. From these data it would appear that approximately 14 to 19% of the applied label that is recovered from the tissue diffuses in a nondirectional manner within the coleoptile during the 3-h period (compare positions A and E with B and C). The data in Table I (position A versus C) indicates that about 14% of the label recovered from the tissue moved laterally within the tissue to the lower side. In contrast to this, position B versus D of Table I shows a weak lateral transport of only about 4%. The much lower lateral transport in position D may be due to the reduced total uptake of IAA into the tissue in this position as shown by the counted radioactivity in Table ID. The total uptake (position D upper) is approximately 65% of that taken up by those in position F (right), whereas the total uptake by those in position B (lower) is 22% greater. This suggests that the tip of the coleoptile may have a role in helping to establish an asymmetric distribution of auxin.

Gravitropic Curvatures of Coleoptiles without Barriers. Parts A and B of Figure 2 illustrate the gravitropic responses of half-decapitated coleoptiles supplied with 3.2 μM IAA for a 3-h period, while Figure 2C illustrates the response of intact coleoptiles given the same gravitational stimulation. In vertical coleoptiles (Fig. 2D), the 3.2 μM concentration can adequately replace the half-decapitated tip resulting in coleoptiles that remain straight until the last hour, as opposed to coleoptiles not supplied with IAA which show a strong curvature in the direction toward the removed half-tip. The slight curvature caused by the addition of IAA may be due to inadequate uptake from the block across a

Table I. *Distribution of Radioactivity from [^{14}C]IAA in Half-Decapitated Coleoptiles*

Distribution of radioactivity in half-decapitated coleoptiles with barriers inserted and oriented with respect to gravity as shown in the table. IAA, 3.2 μM (51 mCi mmol^{-1}), was supplied in 20 mM citrate-KOH (5.65 pH) buffered agar blocks, the average dpm per block was 4120 $\pm$ 121. The distribution was calculated on a per coleoptile basis, with the per cent distribution based on the mean total activity recovered per coleoptile in a given treatment. All values are the mean of four individual experiments with three coleoptiles in each treatment. Capital letters refer to equivalent orientations in Figure 1.

Removed Half-tip and Barrier Position		Coleoptile Section	Distribution CPM$\pm$S.D.	Distribution Percent
	B	Upper	63$\pm$2.5	12.8
		Lower	429$\pm$6.5	87.2
	D	Upper	214$\pm$4.6	81.7
		Lower	48$\pm$2.2	18.3
	A	Upper	305$\pm$5.5	73.1
		Lower	112$\pm$3.3	26.9
	C	Upper	42$\pm$2.0	12.5
		Lower	294$\pm$5.4	87.5
	F	Right	346$\pm$5.9	85.6
		Left	58$\pm$2.4	14.4
	E	Right	74$\pm$2.7	19.0
		Left	315$\pm$5.6	81.0

transverse cut or a slightly enhanced auxin status in the intact half-tip. Figure 2D can be contrasted with Figure 3D which has the same protocol, except 1 μM IAA is supplied. At this lower IAA concentration, there is a steady rate of curvature toward the removed half-tip commencing after 30 min that continues for 2 h, then begins to decrease. This curvature indicates that the IAA concentration is inadequate to replace the half-tip with regard to straight growth.

Of particular importance are the gravitropic curvature responses in relation to the position of the removed coleoptile half-tip when compared at these two concentrations. Figures 2A and 3A show the curvature of coleoptiles when the removed half-tip is in an upward position. The curvature response to these two concentrations is essentially the same. In comparing these IAA responses with those of the intact coleoptiles (Figs. 2C and 3C) and blank controls (Figs. 2A and 3A) all responses are found to be similar in total magnitude and rate. Figures 2B and 3B show the opposite orientation, *i.e.* with the removed half-tip in a downward position. Both concentrations result in a total curvature of about one-half that of the intact coleoptiles but the 3.2 μM IAA-treated coleoptiles achieve that curvature 1 h earlier. Differences in responses to these two applied concentrations may be attributed to differences in the lateral movement of IAA from the intact half-tips. The lower concentration of IAA does nearly as well, in terms of both maximal rate of curvature and total curvature, as the higher concentration, but seemingly is inadequate to maintain straight growth.

Figures 2 and 3 also illustrate the gravitropic responses of partially decapitated coleoptiles supplied with agar blocks that contain no IAA. When the removed half-tip is in an upward position (Figs. 2A and 3A), curvature is nearly identical to that of the intact coleoptiles. Placing the side with the removed tip in the downward position (Figs. 2B and 3B) results in essentially no curvature for the first 1.5 h followed by an increased rate of

upward curvature. Whereas in the downward position, the rate of curvature shows an initial lag followed by an increase during the last hour, in the upward position it appears to closely resemble the intact response. This would indicate that there is a requirement for auxin to support growth in both vertical and gravity-stimulated coleoptiles.

Generally the radioactivity distribution experiments without barriers followed the same trends as those with barriers. The total uptake into the upper half of coleoptiles that had been oriented with removed half-tip in the downward position was less than the uptake into the lower half of coleoptiles that had been oriented in the opposite direction (Table II). In some experiments, the coleoptiles were split longitudinally, then subdivided into equal apical and basal halves. Table II shows the distribution of label within coleoptiles for the concentration 3.2 μM. The pattern of distribution for a 1 μM IAA supply was the same as for a 3.2 μM IAA supply; only the total uptake was different.

The basal one-half of each split coleoptile contained a much smaller percentage of label than the apical one-half as one might have predicted. Less label consistently appeared in the upper basal half than in the lower basal half of gravitationally stimulated coleoptiles. This difference probably is due to lateral transport and/or a decreased longitudinal transport on the upper side, as reported by Cane and Wilkins (1). There appear to be no unusual patterns in the distribution of label. The asymmetry of radioactivity, though small, is most likely the result of specific lateral transport, differential longitudinal transport, and/or a differential uptake depending upon entry surface and orientation. It is assumed that the distribution of label within the tissue reflects the internal distribution of auxin. This, however, may not be the case as the intact coleoptile half-tip will provide a continuous supply of endogenous IAA that may be distributed more efficiently from the tip than from an agar block.

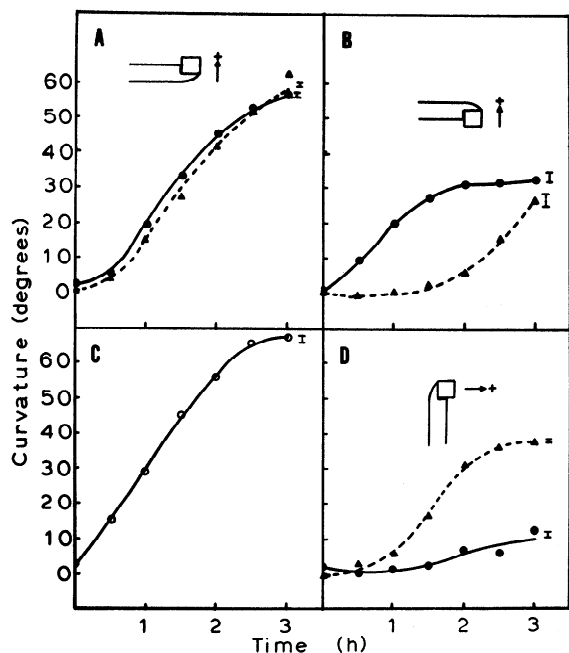**Conclusions.** Unlike the root cap, the coleoptile tip is not the

FIG. 2. Gravitropic responses of half-decapitated coleoptiles supplied with 3.2 μM IAA without barriers. Coleoptiles were reorientated with respect to gravity with either the intact half-tip up or down for the 3-h duration of the experiment. Coleoptiles were selected and treated as in Figure 1, except that barriers were not used. Blank controls ($\blacktriangle$---$\blacktriangle$) containing no added IAA were included for each treatment. Intact sets of coleoptiles (O——O) were included as further controls. Arrows indicate direction of curvature. All points represent the means of three separate experiments. (SEM = ±1.5, the error bar by each curve represents the SD of the individual means.)

FIG. 3. Gravitropic responses of half-decapitated coleoptiles supplied with 1 μM IAA and without barriers. Coleoptiles were reorientated with respect to gravity with either the intact half-tip up or down. Coleoptiles were selected and treated as in Figure 1, except that the IAA concentration was 1 μM and barriers were not used. The corresponding blank controls ($\blacktriangle$---$\blacktriangle$) and intact controls (O—O) were included. (SEM = ± 1.0, the error bar by each curve represents the SD of the individual mean.)

unique site of gravity perception, though it has been suggested to play a unique role in the development of gravitropic curvature (14, 19). The findings of this and earlier work (15) support the contention that the apex may have a special role in the gravitropic response. Shaw et al. (15) observed that the site of application of [³H]IAA on the coleoptile apex affected the asymmetric distribution of the label. The data presented here (Tables I and II) shows that the amount of IAA taken up by the longitudinal cut surface of a half-decapitated coleoptile is dependent upon orientation. If the remaining intact half is oriented in an upward position, uptake is much less than when it is oriented in a downward position. This indicates that the tip, whether it is the site of IAA production, IAA distribution, or both, could be important in helping to establish an asymmetric distribution of IAA upon gravitational stimulation.

The Cholodny-Went theory of gravitropism rests on the premise that IAA is transported laterally in responding tissue and that this lateral movement controls the asymmetric growth pattern that is established (18). Though the lateral transport has been verified in organs such as corn coleoptiles, the total signficance of this movement is unclear. The general debate seems to be concerned mainly with whether sufficient IAA is moved to the lower side of the coleoptile to account for the increased growth observed on that side.

Earlier investigations into responses of corn coleoptiles to IAA (2, 3, 9, 13) indicate that IAA is required for normal growth. The actual quantity, form, and mechanism of action is still not well-defined. Recent work (4, 5, 11) suggests that the coleoptile tissue can adapt to a low level of IAA by increasing sensitivity to it. We have found that corn coleoptiles can also adapt to a high level of auxin, becoming desensitized to it in order to maintain a predetermined growth rate (11). This indicates that the control
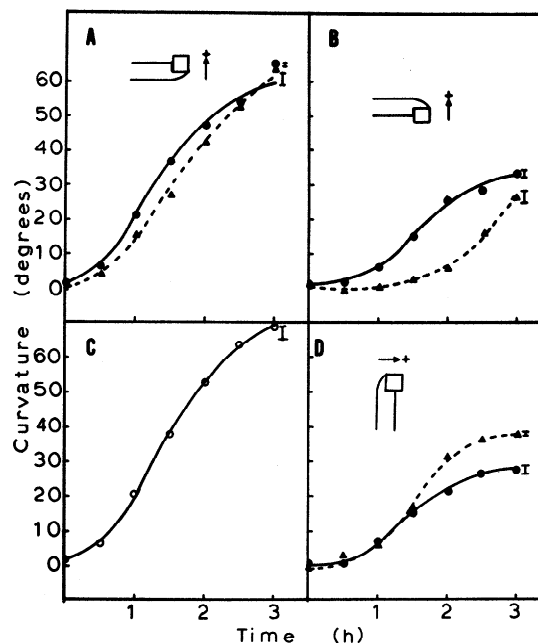
of growth in this tissue is more complex than expected from a simple relationship to the concentration of IAA. It appears to involve, in addition, processes through which the tissue becomes sensitized or desensitized to the auxin present. Which process is evoked seems to depend on auxin concentration. These processes may involve a feedback mechanism altering the sequence of events in the growth-regulating process.

These data suggest that the roles of IAA in the gravitropic response of corn coleoptiles may not be of a single nature as proposed by the Cholodny-Went theory. Since one early aspect of the gravitropic response is the decreased growth rate on the upper side of the horizontal organ (6), IAA moving to the lower side could help to explain this aspect of the response. Though the overall control of the growth rate may be complex, it would seem that over short, steady-state growth periods a specific level of IAA is required. When stimulated by gravity, a lateral movement of IAA from upper to lower side commences all along the coleoptile. This would cause a drop in the effective level of IAA in the upper side, resulting in a much reduced growth rate within that side after a short period of time. The significance of this reduced growth rate on the upper side is often overlooked when considering the total gravitropic response. From the work of Firn and Digby (6), it would seem that the reduced growth on the upper side is as important as, and in some instances more important than, the increased growth on the lower side. Responses of coleoptiles in which the upper portion of the tip is removed and no IAA is supplied (Figs. 1, A and B; 2A; 3A) mimics the intact coleoptile response. This suggests that slowing or stopping the growth rate on the upper side by auxin removal could be an important component of the gravitropic response.

The question still remains as to whether IAA that moves to the lower side is sufficient to stimulate growth. The possibility that a highly localized accumulation of IAA occurs on one side of the coleoptile, e.g. in the epidermis of its lower side or in some other growth-limiting portion of the coleoptile, has not been

Table II. *Regional Distribution of Radioactivity from [¹⁴C]IAA in Half-Decapitated Coleoptiles*

Distribution of radioactivity in half-decapitated coleoptiles was determined after 3-h uptake period for coleoptiles oriented with respect to gravity as shown in the table. Sections were subdivided into apical (including the tip) and basal regions of equal portions. Data shown were for experiments using 3.2×10^{-6} M IAA (51 mCi mmol^{-1}) supplied in agar blocks containing 20 mM citrate-KOH buffer (5.65 pH). The average dpm per block was 4120 ± 121. Experiments using 1.0×10^{-6} M IAA had the same distribution patterns. All values are the mean of three experiments with three coleoptiles in each treatment.

Coleoptile Position	Coleoptile Longitudinal Section	Half Section Region	Distribution CPM±S.D.	%	Total CPM±S.D.	%
	upper	apical	149±3.9	24.6	214±4.6	45.3
		basal	65±2.5	10.7		
	lower	apical	275±5.2	45.5	391±6.2	64.7
		basal	116±3.4	19.2		
	upper	apical	265±5.1	44.6	332±5.8	55.9
		basal	67±2.6	11.3		
	lower	apical	173±4.2	29.1	262±5.1	44.1
		basal	89±3.0	15.0		

satisfactorily excluded. Though this hypothesis cannot be dismissed, it is not strongly supported by these results (*e.g.*, Fig. 1B). Alternatively, it may be concluded that the increased IAA on the lower side is not so important in terms of the interaction with the primary binding site for growth. It could be that, upon gravitational stimulation, a secondary binding site essential to growth is activated or the primary site is modified so that it is more sensitive to the increased total IAA pool within the cells. It has been clearly demonstrated that the coleoptile tissue can adapt to the level of auxin present, becoming more sensitive to low concentrations or less sensitive to high concentrations. Thus, it would seem possible that adjustments in sensitivity to auxin of even shorter time course might occur upon gravitational stimulation. This could account for a rapidly changing growth rate without log-concentration changes in IAA levels.

LITERATURE CITED

1. CANE AR, MB WILKINS 1969 Independence of lateral movement of indoleacetic acid in geotropically stimulated coleoptiles of *Zea mays*. Plant Physiol 44: 1481–1487.
2. CLELAND R 1972 The dosage-response curve for auxin-induced cell elongation: a reevaluation. Planta 104: 1–9
3. DURAND H, MH ZENK 1970 Initial kinetics of auxin-induced cell elongation in coleoptiles. *In* DJ Carr, ed, Plant Growth Substances, 1970. Springer-Verlag, New York, pp 62–67
4. EVANS ML, MR SCHMITT 1975 The nature of spontaneous changes in growth rate in isolated coleoptile segments. Plant Physiol 55: 757–762
5. EVANS ML, M SIMON, M VESPER 1977 Further characterization of the spontaneous growth response in *Zea* coleoptile segments. Plant Cell Physiol 18: 441–452

6. FIRN RD, J DIGBY 1979 A study of the autotropic straightening reaction of a shoot previously curved during geotropism. Plant Cell Environ 2: 149–154
7. FIRN RD, J DIGBY 1980 The establishment of tropic curvatures in plants. Annu Rev Plant Physiol 31: 131–148
8. FIRN RD, J DIGBY, A HALL 1981 The role of the shoot apex in geotropism. Plant Cell Environ 4: 125–129
9. FRENCH RC, H BEEVERS 1953 Respiration and growth responses induced by growth regulators and allied compounds. Am J Bot 40: 660–666
10. GOLDSMITH MHM, MB WILKINS 1964 Movement of auxin in coleoptiles of *Zea mays* L. during geotropic stimulation. Plant Physiol 39: 151–162
11. HATFIELD RD, CE LAMOTTE 1984 IAA-induced growth responses of decapitated corn seedlings: Indications of two apparent adaptations with a possible role in gravitropism. Plant Physiol 74: 304–306
12. NAVEZ AE, TW ROBINSON 1932 Geotropic curvature of *Avena* coleoptiles. J Gen Physiol 16: 133–145
13. RAYLE DL, ML EVANS, R HERTEL 1970 Action of auxin on cell elongation. Proc Natl Acad Sci USA 65: 184–191
14. REINHOLD L 1978 Phytohormones and the orientation of growth. *In* DS Letham, PB Goodwin, TJV Higgins, eds, Phytohormones and Related Compounds: A Comprehensive Treatise, Vol II, Phytohormones and the Development of Plants. Elsevier/North-Holland Biomedical Press, Amsterdam, pp 251–289
15. SHAW S, G GARDNER, MB WILKINS 1973 The lateral transport of IAA in intact coleoptiles of *Avena sativa* L. and *Zea mays* L. during geotropic stimulation. Planta 115: 97–111
16. TREWAVAS A 1981 How do plant growth substances work? Plant Cell Environ 4: 203–228
17. VESPER M, ML EVANS 1978 Time-dependent changes in the auxin sensitivity of coleoptile segments: apparent sensory adaptation. Plant Physiol 61: 204–208
18. WENT FW, KV THIMANN 1937 Phytohormones. The Macmillan Co., New York
19. WILKINS MB 1979 Growth-control mechanisms in gravitropism. *In* W Haupt, ME Feinleib, eds, Encyclopedia of Plant Physiology, New Series, Vol 7, Physiology of Movements. Springer-Verlag, New York, pp 601–626